Cells in 50 μl media
(cells plated the night before)

↓

The next day the plate is left on ice for loading CPA

↓

Add 50 μl 0.50M mannitol to all wells

↓

Remove mannitol/media

↓

Wash wells with 100 μl of final CPA concentration

↓

Add 100 μl of final CPA concentration to appropriate wells

↓

Cool plate at -1.0 °C/min. to -80 °C

↓

Leave plate at -135 °C overnight

↓

Plate is removed from freezer and warmed to ~4 °C;
150 μl 0.5M mannitol/media (warmed to 37 °C) is added during thawing

↓

Put the plate on ice and remove mannitol/CPA

↓

Wash wells 2x with 100 μl 0.5M mannitol

↓

Wash wells 2x with 100 μl DMEM (10%FCS)

↓

Leave 200 μl DMEM (10%FCS) in well for Alamar Blue assay

FIG. 1

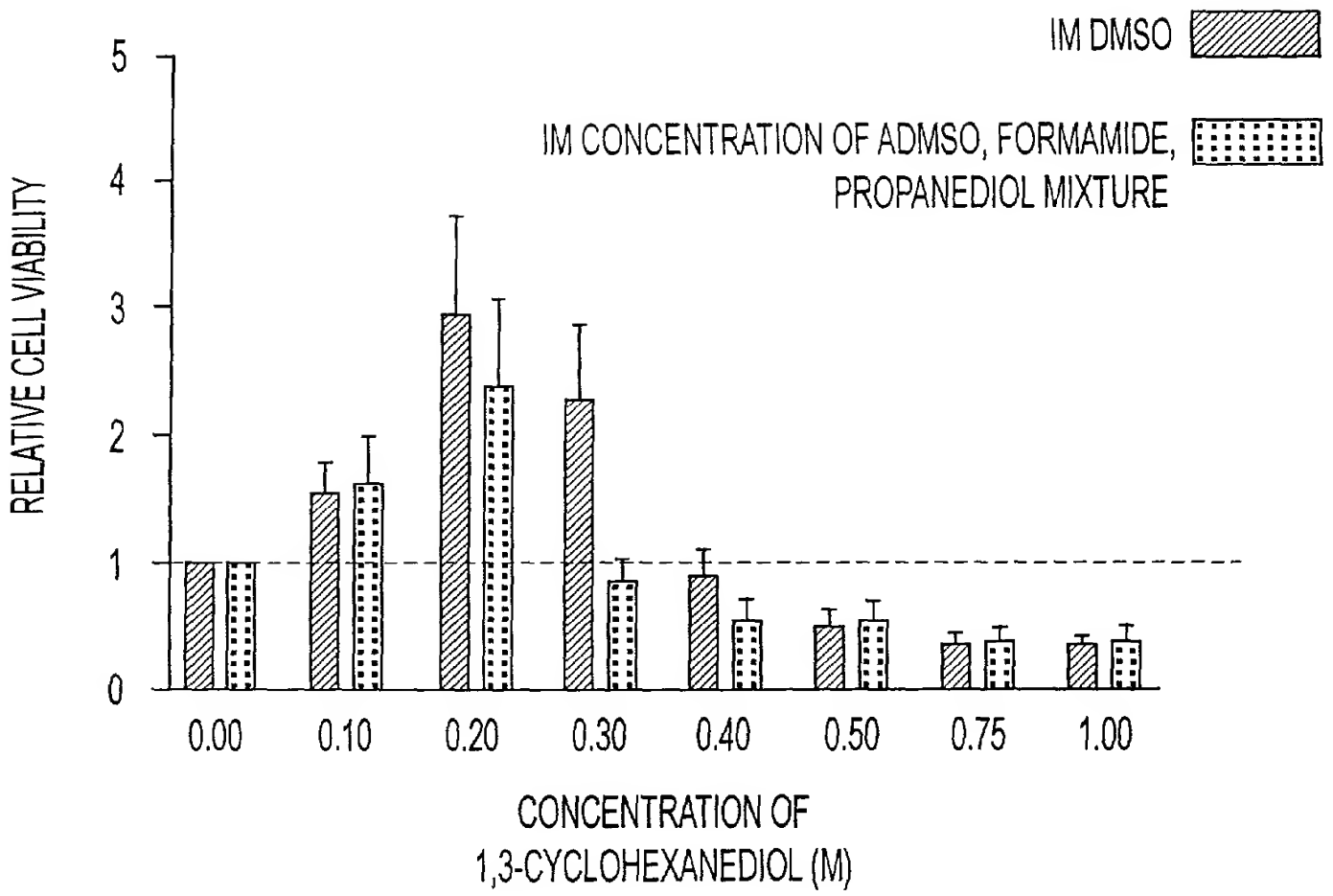
IM DMSO
IM CONCENTRATION OF ADMSO, FORMAMIDE, PROPANEDIOL MIXTURE
RELATIVE CELL VIABILITY
5
4
3
2
1
0
0.00
0.10
0.20
0.30
0.40
0.50
0.75
1.00
CONCENTRATION OF 1,3-CYCLOHEXANEDIOL (M)

FIG. 2

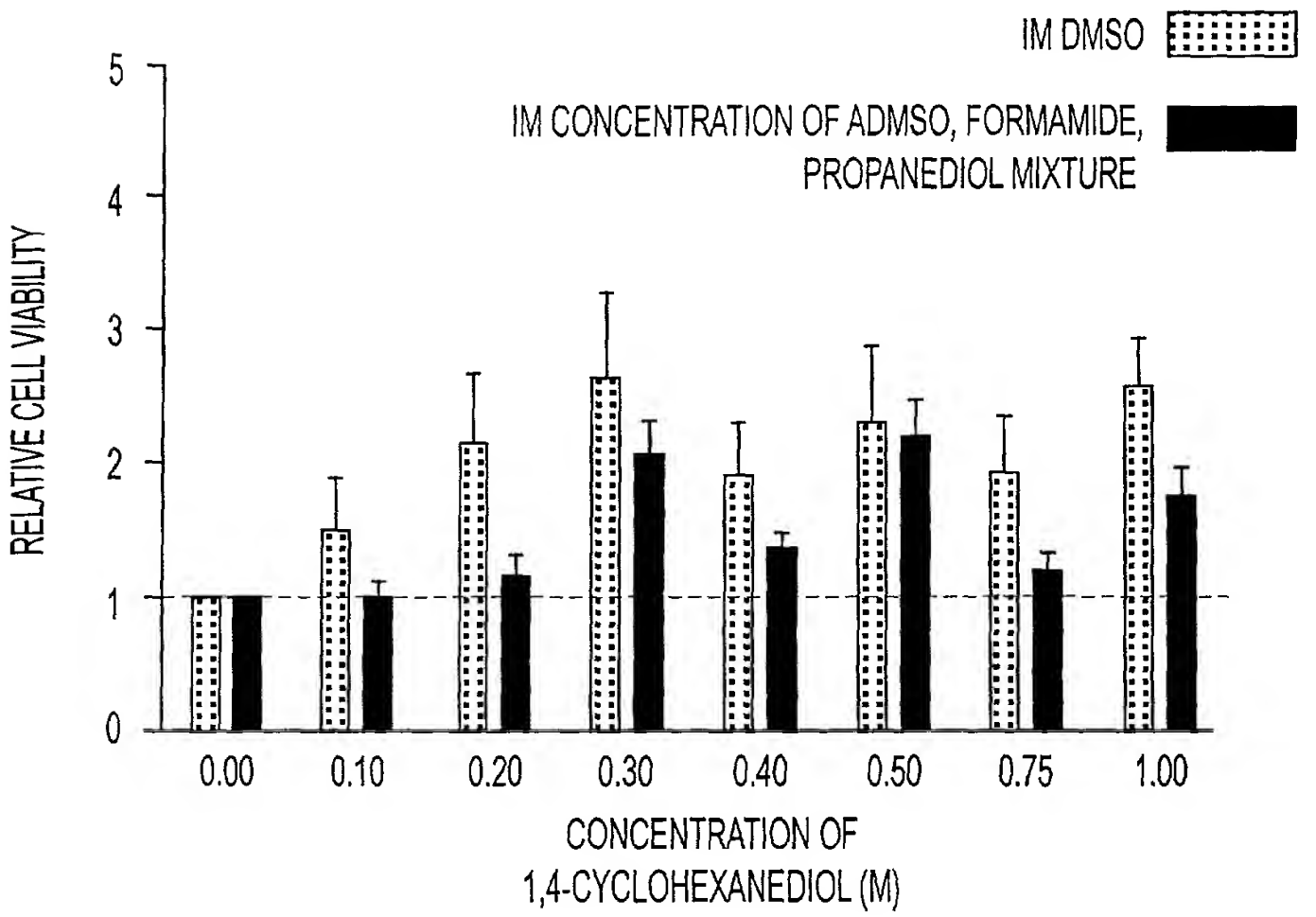
IM DMSO
IM CONCENTRATION OF ADMSO, FORMAMIDE, PROPANEDIOL MIXTURE
RELATIVE CELL VIABILITY
5
4
3
2
1
0
0.00
0.10
0.20
0.30
0.40
0.50
0.75
1.00
CONCENTRATION OF 1,4-CYCLOHEXANEDIOL (M)

FIG. 3

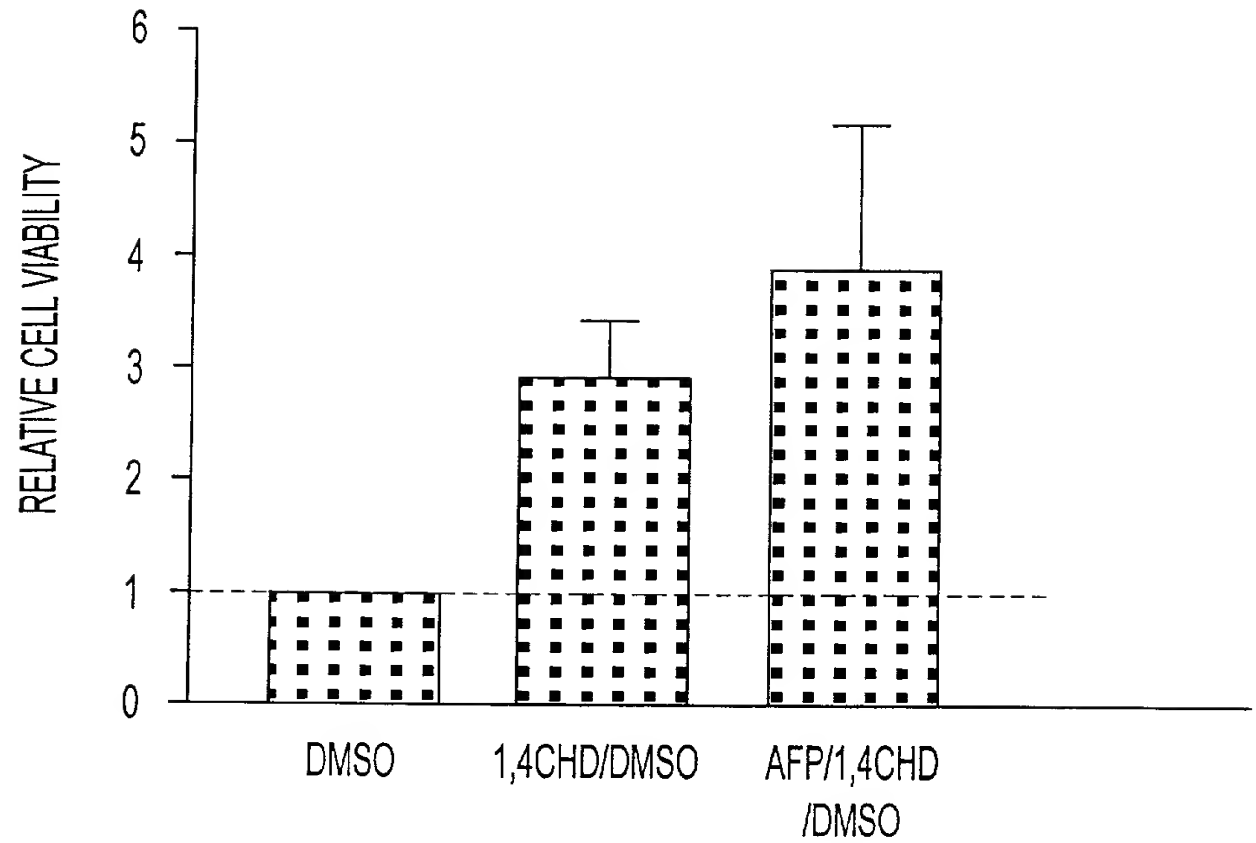
RELATIVE CELL VIABILITY
6
5
4
3
2
1
0
DMSO
1,4CHD/DMSO
AFP/1,4CHD
/DMSO

FIG. 4

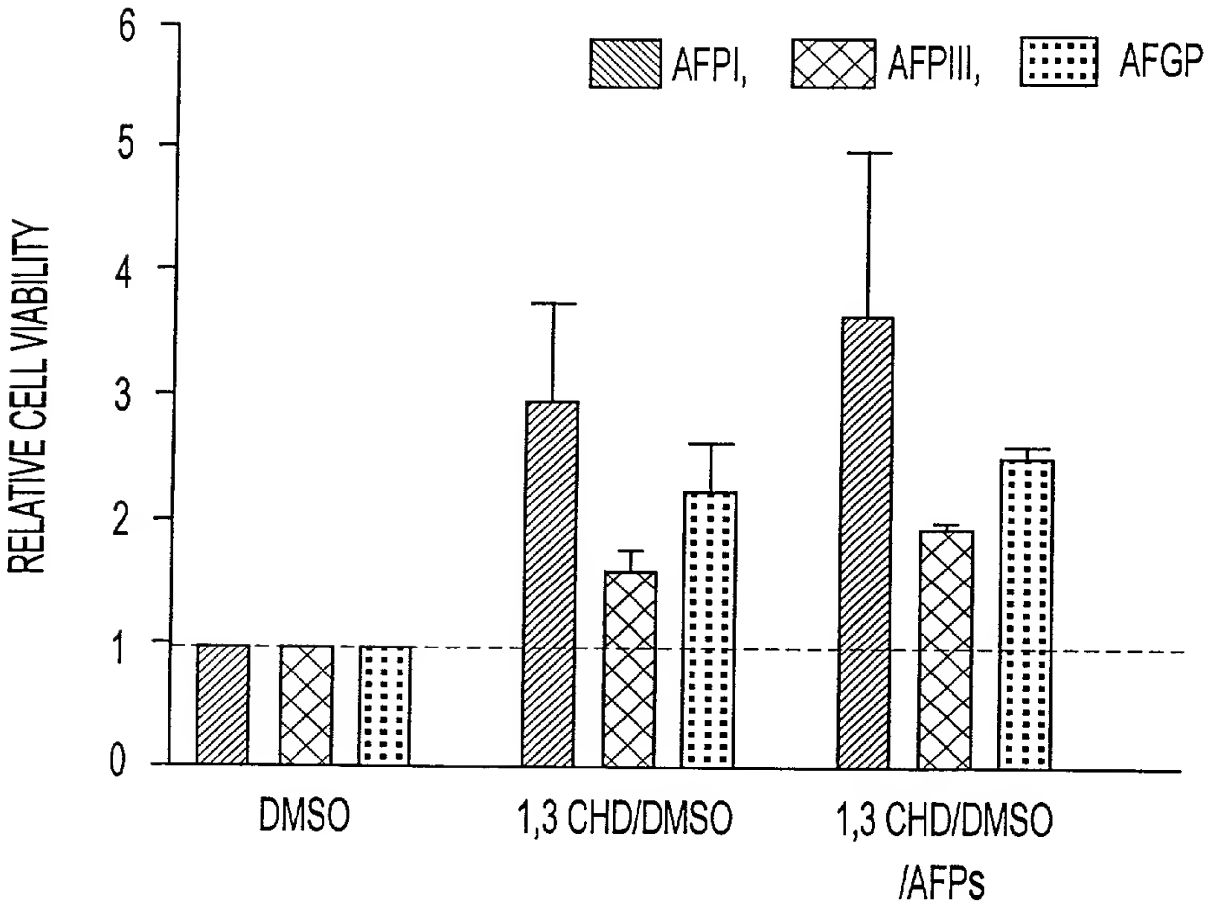
AFPI,
AFPIII,
AFGP
RELATIVE CELL VIABILITY
6
5
4
3
2
1
0
DMSO
1,3 CHD/DMSO
1,3 CHD/DMSO
/AFPs

FIG. 5